REMARKS

Reconsideration and withdrawal of the rejection of the claims in view of the remarks herein is respectfully requested. Claims1-5 are amended. The pending claims are claims 1-12. No new subject matter has been added. The amendments are made to clarify the claims, and not for reasons relating to patentability. Therefore, the amendments are not intended to limit the scope of equivalents to which any claim element may be entitled.

The specification is amended to correct typographical errors.

At page 2, paragraph 3 of the Office Action, the Examiner objected to claims 3-12 under 37 CFR § 1.75(c) as being dependent upon other multiple dependent claims. However, a Preliminary Amendment filed December 16, 1999 amended the claims to remove all multiple dependencies. This objection is therefore rendered moot.

Support for the amendments to claim 1 is found in the specification at page 6, lines 29-31; page 7, lines 14-17; page 8, lines 30-31 and 34; and page 21, lines 24-26.

Support for the amendment to claim 2 is found in the specification at page 7, lines 30-36.

Support for the amendment to claim 3 is found in the specification at page 9, lines 18-25.

Support for the amendment to claim 4 is found in the specification at page 10, lines 7-10; page 11, lines 3-11; and page 12, lines 13-22.

Claim 5 is amended to in response to the Examiner's comments appearing at page 4, paragraph 9 of the Office Action.

The Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1-12 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Examiner asserts that the phrases "the assessment of alcohol consumption" and "carbohydrate-free transferrin" in claim 1 are unclear; the term "derived" in claim 2 is unclear; the phrase "fragments thereof" and "mixtures thereof" in claims 3 and 5 are unclear; and the phrase "different lectins" in claim 4 is unclear. The Examiner also asserts that claim 5 recites improperly expressed alternative limitations. Claim 1 has been amended to no longer recite "the assessment of alcohol consumption"; claim 2 has been amended to no longer recite the term "derived"; and claim 4 has been amended to no longer recite the

phrase "different lectins", thus rendering moot these particular rejections of claims 1, 2 and 4. As these rejections may be maintained with respect to the pending claims, they are respectfully traversed.

It is well-settled that claim language is sufficiently definite if one of ordinary skill in the art would understand the scope of the claim when read in light of the specification. In re Marosi, 710 F.2d 799, 218 U.S.P.Q. 289 (Fed. Cir. 1983); Morton Inst. Inc. v. Cardinal Chemical Co., 28 U.S.P.Q.2d 1190 (Fed. Cir. 1993); Miles Laboratories v. Shandon Inc., 27 U.S.P.Q.2d 1123 (Fed. Cir. 1993), *cert. denied*, 510 U.S. 1100 (1994).

Claim 1, as amended, is directed towards a method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand; separating a carbohydrate-free transferrin containing fraction not binding to said ligand and determining the content of transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample.

With respect to the phrase "carbohydrate-free transferrin" as is recited in claim 1, Applicant's specification discloses that carbohydrate-free transferrin (CFT) refers to transferrin isoforms that are completely devoid of carbohydrate (page 6, lines 28-30). Moreover, "carbohydrate-free" is defined as "any transferrin molecule which has lost both of its carbohydrate side chains and is substantially free of any residual N-linked oligosaccharide moieties" (page 7, lines 14-17). Therefore, the metes and bounds of a claim which recites an "carbohydrate-free transferrin" would be readily understood by the art worker.

It is Applicant's position that the term "derived" in the context of claim 2 and the specification is clear to a person of ordinary skill in the art. Applicant discloses that a blood derived sample is cell-free, e.g., serum or plasma (page 7, lines 34-36). However, to advance prosecution, claim 2 has been amended to recite "sample obtained from blood".

As for claims 3 and 5, the phrase "mixtures thereof" is not indefinite in view of the recitation in the claims and in view of the specification. Claims 3, as amended, and 5 are directed to a method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising contacting a sample

of said body fluid with a carbohydrate-binding ligand, e.g., antibodies or antibody fragments thereof, lectins and mammalian or microbial carbohydrate-binding proteins, or mixtures thereof (claim 3) or *Sambucus nigra* lectin, *Sambucus sielbodiana* lectin, wheatgerm agglutinin, *Maackia amurensis* lectin, *E. coli* K99 lectin, *Helicobacter pylori* lectin, *Ricinus communis* lectin, and *Crotalaria junctae* lectin, and anti-sialic acid antibodies, and mixtures thereof (claim 5). Applicant discloses that one or more carbohydrate-binding ligands, i.e., generally a protein capable of binding to any carbohydrate, oligosaccharide or sugar structures, may be employed to separate CFT from other transferrin variants (page 9, lines 9-18). For example, the carbohydrate-binding protein may be, for example, an antibody, e.g., a monoclonal antibody, a polyclonal antibody, an antibody fragment, single chain antibody, or a lectin "used singularly or in combination with ... other types of carbohydrate binding proteins" (emphasis added, page 10, lines 8-10 and page 9, lines 18-26). Combinations of different carbohydrate ligands are disclosed to have increased binding capacity and hence provide better separation of transferrin isoforms (page 11, lines 3-11). Examples of such combinations are disclosed at page 12, lines 13-22.

Thus, it is clear from the claims and the specification that "mixture thereof" refers to a combination of one or more carbohydrate-binding ligands Hence, one of ordinary skill in the art, in possession of the specification, would be readily apprised of the metes and bounds of "a mixture thereof" in context of the claims.

At page 4, paragraph 9 of the Office Action, the Examiner asserts that claim 5 recites improperly expressed alternative limitations. However, it is Applicant's position that claim 5 properly expresses alternative limitations and is definite. Alternative expressions are permitted if they present no uncertainty or ambiguity with respect to the question of scope or clarity of the claims. M.P.E.P. § 2173.05(h). Support for the language recited in claim 5 can be found, e.g., Appendix AI (PCT) of the M.P.E.P. (see, specifically, Example 20, p. AI-61 to AI-62 of the August 2001 edition). Thus, it is clear that the phrase "X selected from A, B, and C" is proper claim language, and therefore claim 5 should not be rejected under 35 U.S.C. § 112(2) for improper claim language. However, to advance prosecution, claim 5 has been amended.

Therefore, withdrawal of the rejection of the claims under § 112, second paragraph, is respectfully requested.

The Rejection under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 1-12 under 35 U.S.C. § 112, second paragraph, as being incomplete for allegedly missing essential steps. In particular, the Examiner asserts that the claim is missing the following steps : (1) contacting the remaining carbohydrate free transferrin for detection; (2) a detection step which states how the transferrin is detected; (3) a correlation step for assessing alcohol consumption once the content of transferrin is determined. This rejection, as it may be maintained with respect to the pending claims, is respectfully traversed.

Claim 1, as amended, is directed towards a method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand; separating a carbohydrate-free transferrin containing fraction not binding to said ligand and determining the content of transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample.

Regarding (1), above, it is not clear what the Examiner means by a "contact step." However, the Examiner is respectfully requested to consider that it is not necessary to contact the CFT with anything in order to quantitate CFT. As disclosed at page 19, lines 8 to 22 of the specification, the content of transferrin in the non-binding or "CFT containing" fraction may be determined either by directly assessing the transferrin content of the separated fraction, or, alternatively, an indirect determination may be carried out in which the transferrin content of the bound fraction is determined and then subtracted from the total transferrin content of the initial sample in order to find the CFT content. Many different standard procedures are known in the art for carrying out such determination, e.g., ELISA or radio immunoassay techniques, and it is not therefore appropriate to limit the claim to a particular form of CFT determination.

As for (2), above, page 6, lines 28-33, of Applicant's specification discloses that CFT, i.e., transferrin isoforms which are completely devoid of carbohydrate, are an indicator or marker for alcoholism. Any assay known for detecting and/or quantifying transferrin can be used to determine the CFT content of a sample, which Applicant discloses is sufficient for a clinically valuable assessment of alcoholism (page 6, lines 34-38). For example, Applicant discloses that assays such as an ELISA, radioimmunoassay, radioimmodiffusion, rocket

immunoelectrophoresis, or particle-based immunoassay may be employed, or other assays known to the art such as those disclosed in U.S. Patent No. 4,626,355 (page 19, lines 16-35). Applicant discloses in working examples the detection of CFT in physiological samples using anti-transferrin antibodies (Examples 1-5 and 7-8). To satisfy the requirements of 35 U.S.C. §112, first paragraph, it is well-settled that Applicant need not recite every assay which can be used to detect CFT by Applicant's method. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976); Ex parte Dubbs and Stevens, 119 U.S.P.Q. 440 (Bd. App. 1958).

As for (3), above, the Examiner's attention is respectfully directed to page 21, lines 24-26, of the specification. "Correlation" of the amount of CFT is a diagnostic step, not a step of the assay method as claimed. The method of the present invention is simply an assay which allows determination of CFT content to be made. As disclosed on page 21 of the specification, "it may be assumed that the presence of any CFT whatsoever is indicative of alcohol abuse," but this is a matter for the medical practitioner to assess in the context of each individual patient.

Based on the remarks presented herein, it is respectfully submitted that the pending claims are in conformance with 35 U.S.C. § 112, first paragraph. Thus, withdrawal of the rejection of the claims under 35 U.S.C. § 112, first paragraph, is respectfully requested.

The 35 U.S.C. § 102(b) rejection

The Examiner rejected claims 1-3, 6-8 and 10-12 under 35 U.S.C. § 102(b) as being anticipated by Sundrehagen (WO 91/19983). As this rejection may be maintained with respect to the pending claims, it is respectfully traversed.

The standard for anticipation is one of strict identity, and to anticipate a claim for a patent a single prior art source must contain all its elements. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q.2d 90 (Fed. Cir. 1986); In re Dillon, 16 U.S.P.Q.2d 1987 (Fed. Cir. 1990). Furthermore, there must be no difference between the claimed invention and the disclosure, as viewed by a person of ordinary skill in the art. Scripps Clinic & Res. Found. v. Genentech, Inc., 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991).

Sundrehagen discusses a method of separating different transferrin variants, particularly CDT (carbohydrate deficient transferrin) from "normal" carbohydrate-containing variants. In this method, fractionation techniques (such as using the different isoelectric points of the

different transferrin variants) are used to separate these transferrin variants and a binding partner is then used to label the separated variants. However, Sundrehagen does not teach or suggest the use of carbohydrate-free transferrin (CFT). As is disclosed by Applicant, CFT does not contain any carbohydrate-containing transferrin moieties, whereas CDT does. Furthermore, separation of CFT from other transferrin isoforms according to the present invention takes place using carbohydrate-binding ligand to effect separation (as specified in step a) of claim 1). It is not necessary to use a form of fractionation as disclosed by Sundrehagen for the separation. Thus, claims 1-3, 6-8 and 10-12 are novel over Sundrehagen. Therefore, withdrawal of the 35 U.S.C. §102(b) rejection is respectfully requested.

The 35 U.S.C. §103(a) rejections

The Examiner rejected claims 4-5 under 35 U.S.C. § 103(a) as being unpatentable over Sundrehagen in view of Pekelharing et al. (Analytical Biochemistry, 165, 320 (1987)). The Examiner also rejected claim 9 under 35 U.S.C. § 103(a) as being unpatentable over Sundrehagen in view of Dreher et al. (Canadian Patent No. 2,074,345). As this rejection may be maintained with respect to the pending claims, it is respectfully traversed.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed invention. Second, the art must provide a reasonable expectation of success. Finally, the prior art reference must teach or suggest all of the claim limitations. The teachings or suggestions, as well as the expectation of success, must come from the prior art, not applicant's disclosure. M.P.E.P. § 2142.

Sundrehagen, as discussed above, does not teach or suggest the use of CFT for use in the assessment of elevated alcohol consumption. Thus, Sundrehagen does not obviate Applicant's invention.

Pekelaharing et al. do not remedy the deficiencies of Sundrehagen et al. Pekelharing et al. describe a lectin-enzyme immunoassay for determining protein glycosylation. Transferrin sialovariants, such as 4-sialo-transferrin, and isoforms having 3, 2, 1 and 0 sialic acid groups (see left-hand column, page 323 of Pekelharing et al.). These isoforms of transferrin are not the same

or similar to CFT isoforms because CFT isoforms contain no N-glycan chains whereas the sialotransferrins described in Pekelharing et al. contain N-glycan chains in which the number of sialic acid residues varies. Thus, as Pekelharing et al. do not teach or suggest the use of CFT, Pekelharing et al. do not render Applicant's invention obvious.

Therefore, neither Sundrehagen alone or in combination with Pekelharing et al. teach or suggest a method for the determination of CFT in a body fluid for use in the assessment of elevated alcohol consumption. Hence, claims 4-5 of the present invention are unobvious over Sundrehagen and Pekelharing et al.

Dreher et al. makes no mention of CFT whatsoever. Therefore, there is no suggestion that the method of Sundrehagen could be modified to determine the content of carbohydrate-free transferrins as claimed in the present invention. Therefore, claim 9 is non-obvious over Sundrehagen in view of Dreher et al.

Withdrawal of the 35 U.S.C. § 103(a) rejection of the claims is therefore respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-371-2106) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

ERLING SUNDREHAGEN

By his Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 371-2106

Date Sept. 24, 2001 By Ann M. McCrackin
Ann M. McCrackin
Reg. No. 42,858

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 24th day of September, 2001. (Monday)

Name Candis B. Buending

Signature Candis Buending

CLEAN VERSION OF AMENDED SPECIFICATION PARAGRAPHS

ASSAY FOR CARBOHYDRATE-FREE TRANSFERRIN
Applicant: Erling Sundrehagen
Serial No.: 09/464,158

The paragraph beginning at page 13, line 35:

The carbohydrate-binding ligands may be immobilized by binding or coupling to any of the well known solid supports or matrices which are currently widely used or proposed for immobilization or separation etc. These may take the form of particles, sheets, gels, filters, membranes, fibres or capillaries or microtitre strips, tubes or plates or wells etc and conveniently may be made of glass, silica, latex or a polymeric material. Techniques for binding the ligand to the solid support are also extremely well known and widely described in the literature. For example, the carbohydrate-binding ligands used may conveniently be coupled covalently to CNBr-activated SEPHAROSE or N-hydroxysuccinimide activated supports, optionally in the presence of low molecular weight haptens to protect the carbohydrate binding sites on the ligand. Other coupling methods for proteins are also well known in the art.

G1

The paragraph beginning at page 16, line 21:

Separations based on centrifugation and/or filtration are convenient. In a preferred embodiment a centrifuge tube (e.g., Eppendorf tube) and "filter cup" format may be used, and such formats are readily commercially available, for example from Millipore. Thus the sample and carbohydrate-binding ligand may be added to the cup in the tube and allowed to bind. The tube (and cup) is then spun, and the non-binding supernatant collects in the tube. The carbohydrate binding ligand may be such as to induce precipitation of the bound carbohydrate moieties or it may be immobilized, for example as a slurry, e.g., a gel or on particles. In either case, the bound carbohydrate binding fraction is retained in the cup.

G2

The paragraph beginning at page 24, section c:

c. The suspensions are transferred to ULTRA-FREE MC Millipore UFC3 OHV (0.45 μm) filter cups and centrifuged.

G3

The paragraph beginning at page 25, line 8:

d. The suspensions are transferred to ULTRA-FREE MC Millipore UFC3 OHV (0.45 μm) filter cups and centrifuged.

The paragraph beginning at page 25, in Example 3, section b:

b. 0.5 ml of agarose (REACTI-GEL from Pierce Chemical Company, US) with lectin from *Helicobacter communis* isolated according to Lelwala et al., "Isolation of a sialic acid-specific surface haemaglutinin of *Helicobacter pylori* strain NCTC11637", Zblatt 280:93-196, 1993 immobilized on the agarose according to the manufacturer of the "REACTI-GEL " package insert, suspended in a buffer of 20 W TRIS buffer pH = 7.5 comprising 150 mM sodium chloride, and then mixed with each of the serum samples in TRIS buffer (see a. above).

G4

The paragraph beginning at page 25, Example 3, section c::

c. The suspensions are transferred to ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

G5

The paragraph beginning at page 27, line 11::

c. The suspensions are transferred to ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

G4

The paragraph beginning at page 27, line 22:

20 µl of serum sample is mixed with 0.5 ml of a 25% preswollen WHATMAN QA52 anion exchange resin suspended in 20M bis(2-hydro")amino-tris(hydro~ethyl)methane pH = 6.3. The chloride content of the medium is carefully adjusted to separate the desired isotransferrin fractions, and this may be monitored by HPLC or isoelectric focusing. Thereafter, 0.5ml agarose elderberry bark lectin (Vector Laboratories) is added, and the suspension is mixed gently. The suspension is thereafter filtered by centrifugation in a Millipore ULTRA-FREE MC UFC3 OHV filter cup, and the filtrate is collected. 200 µl of the filtrate is mixed with 200 µl of an transferrin antibody (Dako) solution diluted 1:10 in 0.27M TRIS, 4.5% PEG 8000, 4.3 W sodium azide pH = 7.4, and the nephelometric signal is read and interpolated in a standard curve constructed from standards of known concentration of human transferrin.

G7

The paragraph beginning at page 28, Example 7, section c:

A8

c. The suspensions are transferred to ULTRA-FREE MC Millipore UFC3 OHV (0.45 µm) filter cups and centrifuged.

CLEAN VERSION OF AMENDED CLAIM SET

ASSAY FOR CARBOHYDRATE-FREE TRANSFERRIN
Applicant: Erling Sundrehagen
Serial No.: 09/464,158

1. A method for the determination of carbohydrate-free transferrin in a body fluid for use in the assessment of elevated alcohol consumption, said method comprising

(a) contacting a sample of said body fluid with a carbohydrate-binding ligand, to bind any carbohydrate or carbohydrate-containing moieties in said sample to said ligand;

(b) separating a carbohydrate-free transferrin containing fraction not binding to said ligand and

(c) determining the content of transferrin in said fraction and thereby determining the content of carbohydrate-free transferrin in said sample.

2. A method as claimed in claim 1, wherein the sample is blood or obtained from blood.

3. A method as claimed in claim 1, wherein the carbohydrate binding ligand is selected from antibodies or antibody fragments thereof, lectins and mammalian or microbial carbohydrate-binding proteins, and mixtures thereof.

4. A method as claimed in claim 1, wherein in step (a) a panel of more than one type of lectin is used.

5. A method as claimed in claim 1, wherein the carbohydrate binding ligand is selected from the group consisting of *Sambucus nigra* lectin, *Sambucus sielbodiana* lectin, wheatgerm agglutinin, *Maackia amurensis* lectin, *E. coli* K99 lectin, *Helicobacter pylori* lectin, *Ricinus communis* lectin, and *Crotalaria junctae* lectin, and anti-sialic acid antibodies, and mixtures thereof.

6. A method as claimed in claim 1, wherein the separation step (b) is by precipitation, centrifugation, filtration or chromatographic methods.

7. A method as claimed in claim 1, wherein the carbohydrate binding ligand is immobilized.

8. A method as claimed in claim 1, wherein an ion exchange step to remove or deplete carbohydrate-carrying transferrins in the sample is performed prior to step (a).

9. A method as claimed in claim 1, wherein the determination of transferrin content in step (c) is achieved by turbidometric or nephelometric means.

10. A kit for use in a method as defined in claim 1, said kit comprising:
one or more carbohydrate-binding ligands; and
means for the detection of transferrin.

11. A kit as claimed in claim 10, wherein said means for detection of transferrin comprise an anti-transferrin antibody or antibody fragment; and preferably, an opacification enhancer.

12. A kit as claimed in claim 10, further comprising a transferrin solution of known concentration or a set of such solutions having a range of transferrin concentrations.